Reference: *Biol. Bull.*, **141**: 235–246. (October, 1971)

GENETIC VARIATION IN THE MARINE ECTOPROCT *SCHIZOPORELLA ERRATA*

JAMES L. GOOCH AND THOMAS J. M. SCHOPF

Department of Biology, Juniata College, Huntingdon, Pennsylvania 16652 and Department of Geophysical Sciences, University of Chicago, Chicago, Illinois 60637

Organic evolution occurs as genetic changes in populations. However, genetic changes in marine invertebrate animals are not always expressed in the observable phenotype over the short time intervals accessible to the biologist. Conspicuous gene-controlled polymorphisms do occur; *e.g.*, well-documented color polymorphisms of the copepod *Tisbe reticulata* (Battaglia, 1958, 1965), species of the isopod *Sphaeroma* (Bouquet, Levi and Teissier, 1951; West, 1964; Bishop, 1969), and the ascidian *Botryllus schlosseri* (Sabaddin, 1959; Milkman, 1967). But in the vast majority of the 250,000 described species of marine invertebrates characters in the external phenotype controlled by single genes have not been recognized. Thus the study of such material has not substantially contributed to a broad knowledge of the genetic differentiation of marine species. Information on the genetic composition of marine invertebrate species in relation to population size and age structure, mode of reproduction, and ecological factors is just beginning to be obtained.

Recently several studies have been published based on the premise that marker genes useful in genetic studies of marine species can be identified independent of external phenotype, and of breeding experiments, by analysis of variations in proteins (Gooch and Schopf, 1969, 1970; Schopf and Gooch, 1970, 1971, using *Schizoporella errata* and other species of marine ectroprocts; Selander, Yang, Lewontin, and Johnson, 1970, using the arthropod *Limulus;* Manwell and Baker, 1970, using the polychaete *Hyalinoecia* and the pogonophoran *Siboglinum;* and Milkman and Beaty, 1970, using the clam *Mytilus*). This approach utilizes zone electrophoresis on cellulose acetate, starch, or acrylamide media followed by staining for specific proteins. Genetic variability is visualized as variation in protein mobility on the electrophoresis medium. With the use of favorable material genotype and allele frequencies at some polymorphic loci are directly ascertainable.

When a local marine population is sampled, it cannot be assumed that genotypes of a locus will be randomly distributed over the area. Differing fitness values of genotypes in different regimes of current energy, depth, substrate, exposure to light, or biotic association may lead to non-random distributions. Since the larvae of many species remain only hours in the water column, larval settlement in close proximity to parental colonies may yield clumps of like genotypes. Positive assortive fertilization could also lead to this type of clustering.

Schizoporella errata (Fig. 1) is one of the best characterized marine species from a genetic point of view. It is a typical encrusting ectoproct, one of the 3500 living species of the phylum. The brick-red, sessile colonies consist of hundreds to a few thousands of individuals of hermaphroditic yet outbreeding zooids. It is important to emphasize that the larva of this species (and of most ectoprocts) is short-lived (hours) thus greatly limiting the range of gene flow in any single generation. The North American range along the Atlantic Ocean is reportedly

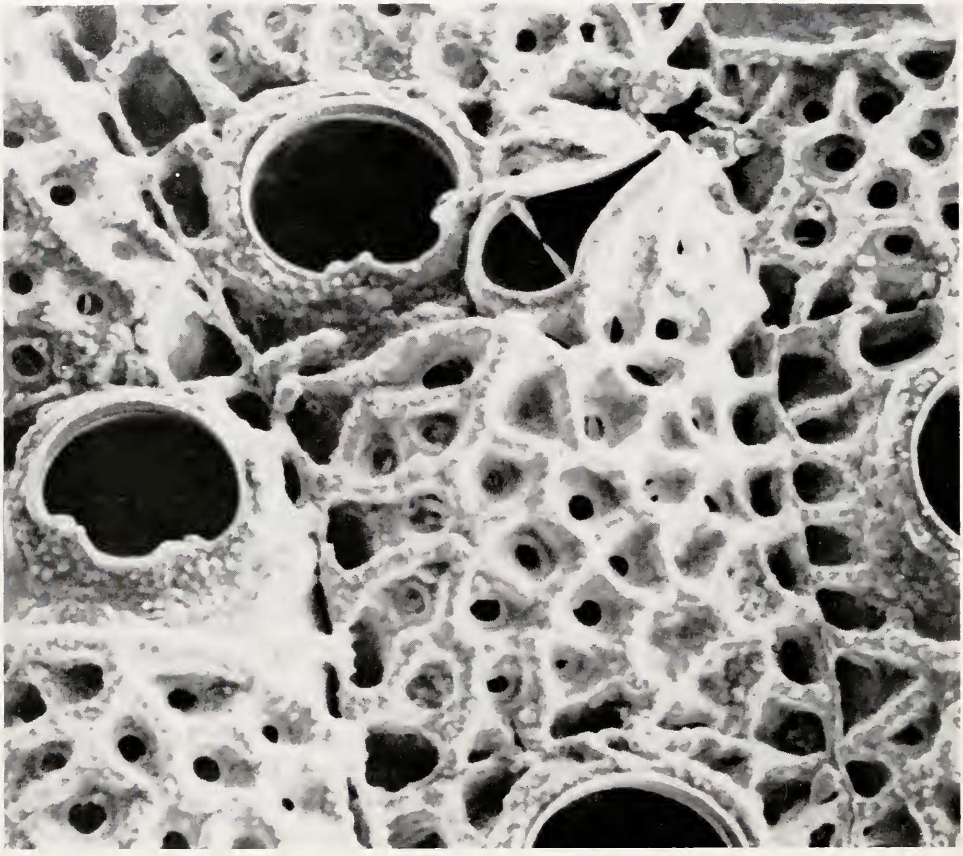

FIGURE 1. Scanning electron microscope photograph of avicularia-bearing individual of a colony of *Schizoporella errata* from Green Pond, Massachusetts. The length of the individual is about 0.6 mm.

from Canada to Florida. *Schizoporella errata* in this paper is apparently the same as *S. unicornis* of all previous literature of the Woods Hole area. Reasons for this purely nomenclatorial change are cited in the detailed descriptions referring to relevant type material and other North American occurrences (Hastings, 1968; Powell, 1970). For purposes of positive identification of the species used in this and previous work, we illustrate here (Fig. 1) a typical specimen. Note the rectangular shape of the zooid, the well-defined sinus of the orifice, the thickened ridges between pores of the frontal surface and the extended (not sharply triangular) avicularium lateral to the orifice.

The fullest interpretation of the data reported here must await a more complete understanding of the size of local populations (now estimated as a few to several hundred colonies extending over tens of m^2), the effect of yearly recruitment (probably substantial at some investigated localities but not at others), and the amount of genetic variability in the rest of the genome. Meanwhile, we wish to present available information and their implications. Results are based on field

collections made in the summer of 1969 (reported in Gooch and Schopf, 1970) and 1970 (presented below).

Materials and Methods

From 13 to 47 colonies of *Schizoporella errata* were collected from pilings, floating docks, and rock jetties at each of 9 stations (Fig. 3) between Cape Cod, Massachusetts, and Beaufort, North Carolina, during the summer of 1970. Except at Cape Cod Canal and Indian River Inlet, the positions of colonies were mapped *in situ* prior to collecting. The majority of collections were from vertical pilings at the localities near Woods Hole. Pilings were classified as "exposed" or "unexposed" depending upon whether they confronted the full force of currents or were shielded from the force of currents by other pilings, rocks, or the local configuration of the sea shore. Furthermore, exposed pilings were partitioned into "protected" and "unprotected" sides by field determination of the direction of principal currents. Colonies were obtained from 0.5 to 3.0 m below low tide as indicated by the barnacle-line.

Collections awaiting electrophoresis were maintained alive in running seawater or were frozen at −60° C for up to three weeks. Freezing had no discernible effect on enzyme patterns. Material was electrophoresed on acrylamide gel using apparatus manufactured by E. C. Corporation (Philadelphia, Pennsylvania).

Procedures for electrophoresing and staining esterases, malate dehydrogenase, and "leucine" aminopeptidase were as previously described (Gooch and Schopf, 1970). Two new enzymes, alkaline phosphatase and tetrazolium oxidase, have been added to systems under analysis. Electrophoresis for alkaline phosphatase was conducted in 0.09 M Tris and 0.015 M boric acid buffer, pH 8.9, for 2½ hours. Gels were incubated in 125 ml 0.04 M Tris and 0.048 N HCl adjusted to pH 7.2 for 3 hours at room temperature. The staining mixture consisted of 50 mg sodium alpha-napthyl phosphate, 50 mg Fast Blue RR Salt, 350 mg polyvinylpyrolidone, 100 mg NaCl, and 0.1 ml of 10 per cent $MgCl_2$. The technique for staining tetrazolium oxidase is similar to that for malate dehydrogenase (Gooch and Schopf, 1970) except that substrate was omitted and development took place in full light. Formazan salts densely stain the gel in areas not occupied by tetrazolium oxidase.

Throughout the Cape Cod to Beaufort transect, an aggregate of 250 colonies of *S. errata* were analyzed for "leucine" aminopeptidase, 91 colonies for esterases, 49 for alkaline phosphatase (Green Pond and Duke Marine Laboratory not sampled), and 18 for tetrazolium oxidase (data for Cuttyhunk, Indian River Inlet, and Shark Shoal Jetty).

The methodology of identifying genetic loci in the absence of breeding tests parallels the approach used by Selander, Yang, Lewontin, and Johnson (1970) for work on *Limulus*, and has been extensively discussed previously (Gooch and Schopf, 1970).

To facilitate statistical treatment of material from localities in the Woods Hole region, adjacent collecting stations that do not show significant differences in gene frequencies were pooled. Thus, as is shown later, the Marine Biological Laboratory (MBL) dock and Sheep Pen Harbor populations (74 colonies) were pooled, as were populations from Robinsons Hole and Cuttyhunk (66 colonies).

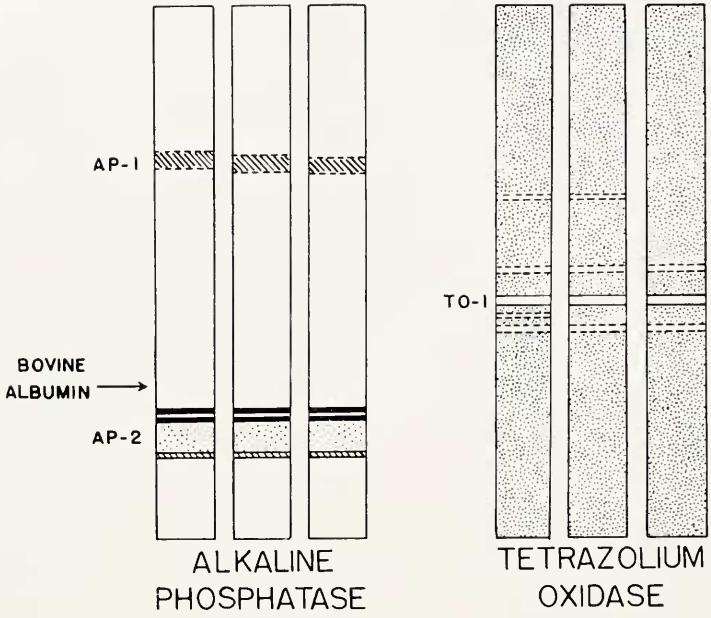

FIGURE 2. Diagram illustrating patterns of banding in *Schizoporella errata* for alkaline phosphatase and tetrazolium oxidase. For alkaline phosphatase, dark bands represent heavily-stained zones of enzyme activity, hatched bands stand for more lightly stained zones, and stippled bands represent faint zones. For tetrazolium oxidase, the clear band represents no staining, and dashed bands represent light staining.

RESULTS

New gene loci

Characteristics of 8 loci were previously documented (Gooch and Schopf, 1970) and these are not different in the material discussed here. Three additional loci, 2 governing alkaline phosphatase patterns, and 1 for tetrazolium oxidase, are newly defined (Table I), making a total of 11 loci characterized for *Schizoporella errata.*

Alkaline phosphatase. Alkaline phosphatase stains as two band systems on acrylamide gel. The enzyme band of the locus Ap-1 averages 0.42 in mobility relative to a Bovine serum albumin (Nutritional Biochemicals Corp.). Resolution in buffer systems tried to date is too poor to establish whether the locus is polymorphic or not.

The second locus, Ap-2, yields 3 closely-spaced and well-resolved bands (Fig. 2). It is monomorphic in all sampled populations for the same allele, designated as $Ap\text{-}2^{1.10}$.

Tetrazolium oxidase. Distinct zones that do not precipitate formazan staining from the decomposition of tetrazolium salts in strong light are often designated as "achromatic zones." These zones have the character of indophenol oxidase in human erythrocytes (Brewer, 1967). The biochemical properties and *in vivo* catalytic activities of the enzyme giving rise to achromatic zones in *S. errata* have

not been investigated; we therefore prefer the operational term "tetrazolium oxidase," following Baur and Schorr (1969).

There is a single zone of tetrazolium oxidase activity, the product of the locus To-1 (Fig. 2). The zone consists of one major band and 2–4 faint leading and trailing bands. Uniform mobility in all populations sampled is indicative of monomorphism for a single allele, here designated To-$1^{0.78}$.

Local distribution of gene frequencies and genotypes at the Lap-3 locus

The Lap-3 locus is an autosomal gene segregating for two codominant alleles, Lap-$3^{0.94}$ and Lap-$3^{0.98}$ in populations near Woods Hole (Gooch and Schopf, 1970). Since it is the sole polymorphic locus in the majority of sampled populations, it is uniquely suitable for analysis of allele frequencies and genotype distribution in local populations.

Effect of depth. Depth is a negligible factor at Green Pond, where most colonies were taken from a floating dock. In the pooled MBL-Sheep Pen Harbor populations 39 colonies were obtained within 1 m of the low tide line, and 35 colonies in the next 2 meters. Gene frequency does not differ significantly in depth comparisons (0.94 allele = 0.55 at 0–1 m, 0.63 at 1–3 m; $\chi^2 = 0.9$, $P > 0.30$). There is, however, a significant excess of heterozygotes in the 0–1 m interval (27 of 39 colonies; $\chi^2 = 6.2$, $P < 0.02$). Genotype distribution between 1–3 m agrees with a Hardy-Weinberg distribution ($\chi^2 = 0.02$, $P > 0.80$).

Since no colonies in the pooled Robinsons Hole-Cuttyhunk populations were taken above 1 m, comparisons are drawn between 1–1.5 m (48 colonies), and 1.5–3.0 m (17 colonies); (one colony on a nearby rock not included). Gene frequency is nearly identical in both depth zones (0.94 allele = 0.32) and genotype distribution in both zones conforms to Hardy-Weinberg equilibrium (1.0–1.5 m, $\chi^2 = 1.9$, $P > 0.10$; 1.5–3.0 m, $\chi^2 = 1.3$, $p > 0.30$).

In 1969 collections, the 0.98/0.98 homozygotes appeared to increase with depth (Gooch and Schopf, 1969, Fig. 5) at the pilings of the MBL dock. Two of the 4 homozygotes occurred at depth less than 2 m ($n = 36$), and 2 occurred at depth greater than 2 m ($n = 4$). In 1970 collections, 3 of the 4 homozygotes occurred at depths less than 2 m ($n = 41$), and 1 occurred at depths greater than 2m ($n = 4$). If it is assumed that the population has remained uniform genetically both years the data may be pooled to give a significant excess of 0.98/0.98 homozygotes in the deeper water. (2×2 table, Yates' correction; $\chi^2 = 4.9$, $P < 0.05$).

Effect of exposure to wave action. The pooled MBL dock-Sheep Pen Harbor populations contained 38 colonies on exposed pilings, 20 protected and 18 unprotected, and 36 colonies on unexposed pilings. Pooled Robinsons Hole-Cuttyhunk populations consisted of 52 colonies on exposed pilings, 31 protected and 21 unprotected, and 14 on unexposed pilings. There are no significant differences in gene frequencies in comparisons of exposed and unexposed pilings (MBL dock-Sheep Pen Harbor, $\chi^2 = 0.06$, $P > 0.80$; Robinsons Hole-Cuttyhunk, $\chi^2 = 0.16$, $P > 0.60$). Nor are there any significant differences in comparisons of protected and unprotected sides (MBL dock-Sheep Pen Harbor $\chi^2 = 0.1$, $P > 0.80$; Robinsons Hole-Cuttyhunk, $\chi^2 = 1.3$, $P > 0.20$).

Genotype distributions on exposed versus unexposed pilings, and protected versus unprotected sides of pilings were compared with expected Hardy-Weinberg

genotype distributions. All comparisons are consistent with Hardy-Weinberg values except unexposed pilings at MBL dock-Sheep Pen Harbor, where the heterozygote class is predominant ($\chi^2 = 6.4$, $P < 0.02$), and the protected sides of exposed pilings at Robinsons Hole-Cuttyhunk, where the heterozygote class is significantly small ($\chi^2 = 4.6$, $P < 0.05$).

Effect of time. Two localities, Green Pond and the MBL dock, were mapped and sampled during both 1969 and 1970 (Fig. 4). Gene frequency at Green Pond for the 0.94 allele was 0.76 (n = 43) in 1969 and also 0.76 (n = 47) in 1970. At the MBL dock, the frequency of the 0.94 allele was 0.72 (n = 50) in 1969 and 0.61 (n = 45) in 1970. The difference is not significant ($\chi^2 = 2.5$, $P > 0.15$). Thus no significant temporal change in over-all gene frequency has been detected.

The 1969 collections yielded evidence of genotypic differences at Green Pond (Gooch and Schopf, 1970). The boat mooring side of the floating dock was occupied by a significant excess of heterozygotes compared to the less polluted open harbor side ($\chi^2 = 4.3$, $P < 0.05$). In the 1970 sample, only 8 colonies were obtained from the mooring side. As in 1969, there was an excess of heterozygotes on the mooring side, with 37 per cent heterozygous colonies as compared with 22 per cent heterozygotes on the open harbor side (Fig. 4). However, the difference in 1970 does not approach statistical significance (homozygous classes combined in 2×2 table, $\chi^2 = 0.7$, $P > 0.30$).

Genetic variation in S. errata

Table I lists 11 defined loci. Eight are well-established as predominantly monomorphic in populations and one, Lap-3, as chiefly polymorphic. The remaining 2 loci are not usable in all populations; Ap-1 because band variation is not interpretable, and Lap-2 because the protein often stains too faintly for analysis. Mobility variation for the E-3 locus in the Cape Cod Canal population is strongly

TABLE I

Gene loci defined in Schizoporella errata *together with allele nomenclature for populations sampled in the Virginian Faunal Province. Note that E-3 has 2 alleles at the Cape Cod Canal (southern border of Acadian Fauna Province), and that Lap-3 has 1 allele in the Carolinian Faunal Province*

Enzyme system	Locus	Number of alleles	Designation of alleles
(1.) Esterase	E-1	1	$E\text{-}1^{1.10}$
	E-2	1	$E\text{-}2^{.66}$
	E-3	1	$E\text{-}3^{.75}$
	E-4	1	$E\text{-}4^{1.18}$
(2.) Malate dehydrogenase	M-1	1	$M\text{-}1^{.99}$
(3.) "Leucine" aminopeptidase	Lap-1	1	$Lap\text{-}1^{.31}$
	Lap-2	1	$Lap\text{-}2^{.68}$
	Lap-3	2	$Lap\text{-}3^{.94}$
			$Lap\text{-}3^{.98}$
(4.) Alkaline phosphatase	Ap-1	1–2 (?)	$Ap\text{-}1^{.42}$
	Ap-2	1	$Ap\text{-}2^{1.10}$
(5.) Tetrazolium oxidase	To-1	1	$To\text{-}1^{.78}$

TABLE II

Summary of gene and genotype distributions for the Lap-3 locus for Schizoporella errata. *MBL means Marine Biological Laboratory; DML means Duke Marine Laboratory*

Locality	Number of colonies	Gene frequency		Genotype (N)		
		Low mobility allele	High mobility allele	Low mobility homozygotes	Hetero-zygotes	High mobility homozygotes
Cape Cod Canal	15	0.80	0.20	10	4	1
Green Pond	47	0.76	0.24	28	15	4
MBL Dock	45	0.61	0.39	14	27	4
Sheep Pen Harbor	29	0.62	0.38	10	16	3
Robinsons Hole	36	0.31	0.69	4	14	18
Cuttyhunk	30	0.35	0.65	6	9	15
Indian River Inlet	13	0.81	0.19	8	5	0
DML Dock	15	1.00	0.0	15	0	0
Shark Shoal Jetty	20	1.00	0.0	20	0	0

suggestive of a second polymorphism. Since this locus shows no variability in the other populations it is assigned overall as monomorphic.

Thus the amount of polymorphism stands at 1 of 9 well-established loci (11 per cent) in the majority of populations and 2 of 9 (22 per cent) in the Cape Cod Canal population. In a few Cape Cod populations Lap-2 is distinguishable as monomorphic. There 1 of 10 (10 per cent) loci are polymorphic.

Regional genetic variation

Throughout the Cape Cod to Beaufort transect 7 loci are uniformly monomorphic for the same alleles. The E-3 locus, as explained above, appears to be polymorphic in the Cape Cod Canal population and is definitely monomorphic in other populations.

The general low level of genetic variation contrasts with the Lap-3 locus, which is polymorphic for the 0.94 and 0.98 alleles in every sampled population north of Cape Hatteras (Table II). Allele frequencies vary widely in segregating populations. The 0.98 allele increases from 0.24 at Green Pond to 0.69 at Robinsons Hole paralleling a local southwestward cooling of summer water temperatures. Regionally, the 0.98 allele decreases from 0.39 at the MBL dock to 0.19 at Indian River Inlet and to 0.0 at DML dock and Shark Shoal Jetty (Fig. 3). This parallels a regional increase in summer water temperatures. Both trends, as well as the increase in the frequency of 0.98/0.98 homozygotes in the deeper, cooler water at MBL dock, suggest selection against the 0.98 allele under warmer conditions. Koehn (1969, 1970) attributed a clinal distribution of allele frequencies at an esterase locus in Catostomid fishes to temperature dependent differences in enzyme activity. Details of the Green Pond-Cuttyhunk transect are presented elsewhere (Schopf and Gooch, 1971).

The Lap-$3^{0.98}$ allele has a frequency of 0.20 in the sampled population from Cape Cod Canal. Animals living in the canal are subject to *daily* fluctuations in temperature of up to 11° C in the late summer (when the material was collected). This remarkably large daily temperature change is due to the water in

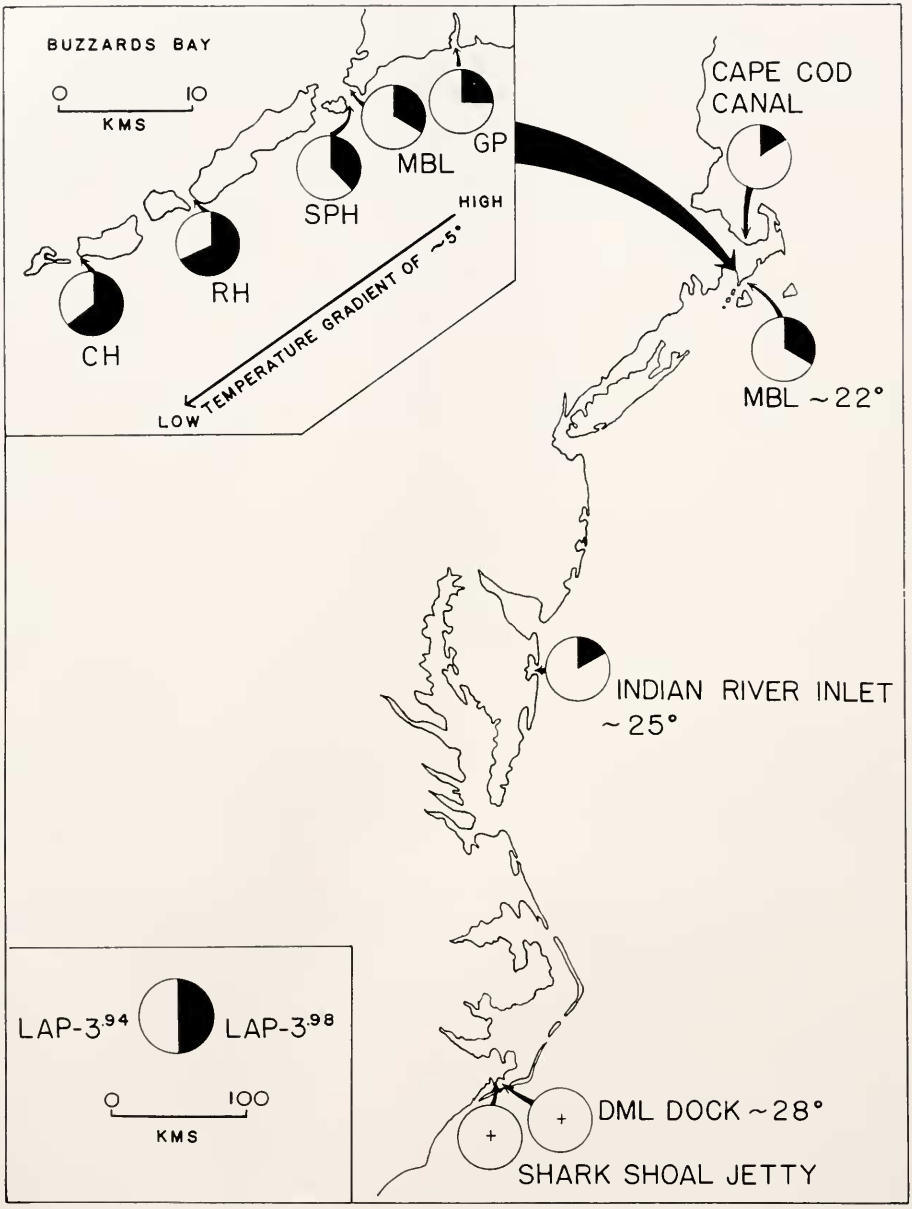

FIGURE 3. Map showing location of 9 collecting localities together with gene frequencies for Lap-3 alleles. Temperatures cited are for the warmest time of the year (late August), and are chiefly meant to emphasize the trend in water temperatures and the relative difference between localities. Note that the frequency of the Lap-$3^{0.98}$ allele decreases as temperature increases in both the Cuttyhunk (CH) to Green Pond (GP) transect and the coastal transect. See text for further discussion. MBL stands for Marine Biological Laboratory dock; SPH for Sheep Pen Harbor; RH for Robinsons Hole; DML for Duke Marine Laboratory.

the canal alternately filling with cold water from Cape Cod Bay and warmer water from Buzzards Bay, depending upon the tidal phase (Administrative-Technical Advisory Committee, 1968; Fairbanks, Collins and Sides, 1968). *S. errata* is apparently dormant during the winter when, in fact, large temperature differences do not exist between these bodies of water. Differential selection with respect to temperature or correlative factors would appear to operate on the Lap-3 locus only during the highest environmental temperatures which are typical of late summer. Thus it is not surprising that allele frequencies indicate selection for individuals able to live in very warm water even though the locality is on the southern edge of the Acadian Faunal Province.

Discussion

Local distributions of allele frequencies and genotypes

The majority of tests of gene and genotype variation with depth, current exposure, and time have revealed no evidence for non-random or temporally changing distributions. Of 17 local distributions 4 have been found which differ significantly from expected values. There was (1) an excess of heterozygotes in the upper 1 m at MBL dock-Sheep Pen Harbor, (2) an excess of the 0.98/0.98 homozygote class in deeper sampling of the MBL dock-Sheep Pen Harbor pilings, (3) an excess of heterozygotes on unexposed pilings at MBL dock-Sheep Pen Harbor, and (4) a deficiency of heterozygotes on the protected sides of exposed pilings at Robinson Hole-Cuttyhunk. It should be remarked that deviations from expected values never strikingly large (P always greater than 0.01).

The excess of heterozygotes in shallowest water may indicate that heterozygotes have superior tolerance to the relatively more variable zone just below the surface. A heterozygote advantage is also a possible explanation for the heterozygote excess found in 1969 at Green Pond on the side of the floating dock nearest the boat moorings (Gooch and Schopf, 1970).

The excess of the Lap-$3^{0.98}$ allele with depth is interpretable in terms of selection for this allele in cooler (deeper) water. However this association with temperature is much more convincingly developed with the local and regional distribution of the Lap-3 alleles. We presently have no evidence that temperature, itself, rather than an unknown environmental factor operating in a temperature-dependent manner, is the direct agent of selection.

The excess of heterozygotes on unexposed pilings, and the deficiency of heterozygotes on protected sides of exposed pilings appear to be contradictory results. We cannot explain these observations based on present information.

Regional genetic variation

The general outlines of the regional genetic differentiation of *Schizoporella errata* can now be drawn. Nine well-characterized gene loci were surveyed in a transect which includes 3 zoogeographic provinces (Johnson, 1934; Cerame-Vivas and Gray, 1966). The Cape Cod Canal population borders the Boreal or Acadian Province, the remaining Cape Cod populations and the Indian River Inlet population are located in the Virginian Province, and the Beaufort populations are in the Carolinian Province. The estimated number of polymorphic loci declines

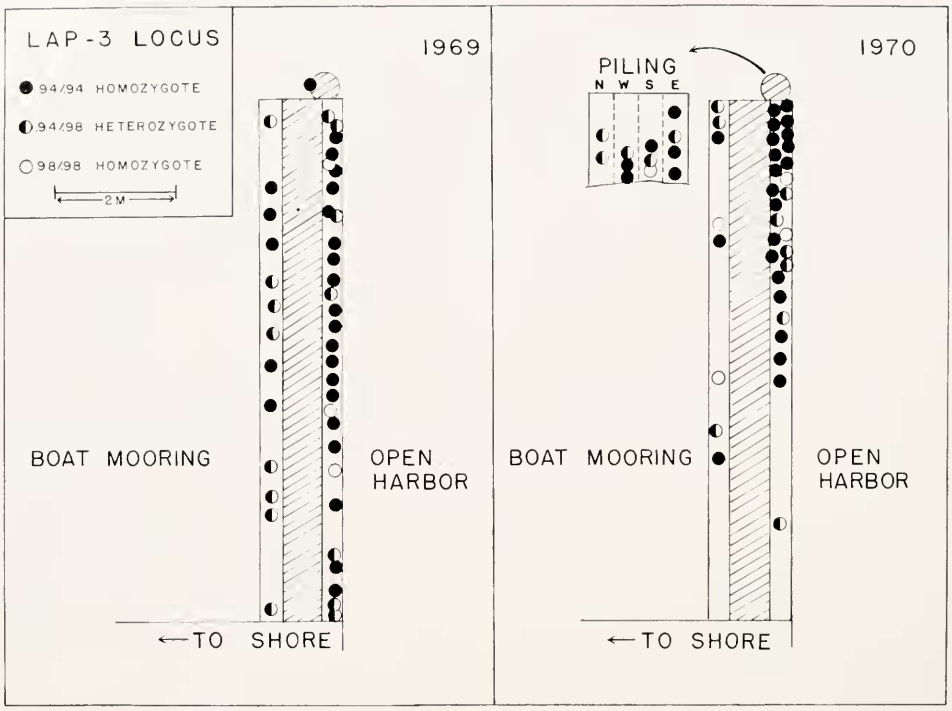

FIGURE 4. Distribution of genotypes of "leucine" aminopeptidase (locus Lap-3) in colonies of *Schizoporella errata* occurring on outer floating dock of boat moorings at Green Pond, Massachusetts. White area on floating dock represents area available for colonization.

southward from 2 at Cape Cod Canal (Lap-3 and E-3) to 1 (Lap-3) in populations of the Virginian Province, and 0 in the populations of the Carolinian Province. Samples are small and data from additional protein systems (and more loci) are much needed to determine if southward diminution of genetic variability is real.

On a regional scale, two major conclusions follow from the pattern of gene frequencies at the diallelic Lap-3 locus: (1) the geographic scale of genetic differentiation may be only a few km since populations separated by 13 km can differ significantly in allele frequency (this aspect is explored fully for populations in the vicinity of Woods Hole in Schopf and Gooch, 1971); and (2) the trend of allele frequencies from Cape Cod to Beaufort approximates a cline, suggesting a regional pattern of natural selection rather than control of gene frequencies by purely local selection pressures or random drift.

On the other hand, the uniform monomorphism of 7 loci throughout the transect, and 8 south of the Cape Cod Canal, is a striking feature of the genome of *S. errata*. Thus 82–89 per cent of the sampled genome is without intra- and interpopulation genetic variation. The occurrence of the same "best allele" at the majority of sampled loci in populations from 3 zoogeographic provinces suggests that *S. errata* is a genetically close knit species, and that adaptation may be primarily regional rather then local. Apparently single alleles have arisen at a sub-

stantial number of loci whose protein products function effectively under the varied conditions throughout the coastal transect. This lack of genetic variation is all the more interesting, since the very limited larval life suggests little gene flow along the full range of the species.

Technical assistance was given by Mr. Kenneth Lindroth, Miss D. L. Knupp and Mr. K. W. Kaufmann, Jr. We thank H. B. Steinbach, D. Clark, and D. Rhoades for the use of small boats for collecting. Discussion with R. G. Johnson was appreciated. The scanning electron microscope photograph (Fig. 1) is by the courtesy of Dr. Virginia Peters, Woods Hole Oceanographic Institution. Work was supported by equipment and research funds granted to James L. Gooch by Juniata College and the Carthage Foundation and by a grant from the Block Fund, University of Chicago, to Thomas J. M. Schopf.

Summary

Schizoporella errata is sessile and has larvae that live only hours. On a purely local scale, the spatial distribution of gene frequencies and genotypes appears random in most comparisons. In addition, genotypes and gene frequencies remained relatively stable after the passage of a year. Over a distance of 1000 km from the southern edge of the Acadian Faunal Province through the Virginian Faunal Province and into the Carolinian Faunal Province, from 80 to 89 per cent of the sampled genome is identical. That is, genetic polymorphism stands at 1 of the 9 well-established loci (11 per cent) and an estimated 2 of 10 total loci in pooled material from 9 localities. The proportion of alleles at the single, clearly polymorphic locus (Lap-3) varies directly as a function of environmental temperature measured at the warmest time of the year.

LITERATURE CITED

Administrative-Technical Advisory Committee, 1968. Biological investigations on the canal electric company's steam-generating plant, Cape Cod Canal, Sandwich, Massachusetts; Interim Report. Unpublished report, 8 pp. [Obtained from Massachusetts Department of Natural Resources, Division of Marine Fisheries, Boston, Massachusetts.]

Battaglia, B. 1958. Balanced polymorphism in *Tisbe reticulata*, a marine copepod. *Evolution*, **12**: 358–364.

Battaglia, B., 1965. Advances and problems of ecological genetics in marine animals. *Proc. 11th Int. Congr. Genet.*, **2**: 451–463.

Baur, E. W., and R. T. Schorr, 1969. Genetic polymorphism of tetrazolium oxidase in dogs. *Science*, **166**: 1524–1525.

Bishop, J. A., 1969. Changes in genetic constitution of a population of *Sphaeroma rugicauda* (Crustacea: Isopoda). *Evolution*, **23**: 589–601.

Bocquet, C., C. Levi and G. Teissier, 1951. Recherches sur le polychromatisme de *Sphaeroma serratum*. *Arch. Zool. Exp. Gen.*, **87**: 245–297.

Brewer, G. J., 1967. Achromatic regions of tetrazolium stained starch gels: inherited electrophoretic variation. *Amer. J. Human Genet.*, **19**: 674–680.

Cerame-Vivas, M. J., and I. E. Gray, 1966. The distributional pattern of benthic invertebrates of the continental shelf of North Carolina. *Ecology*, **47**: 260–270.

Fairbanks, R. B., W. S. Collings and W. T. Sides, 1968. Biological investigations in the Cape Cod Canal prior to the operation of a steam generating plant. Unpublished report, 116 pp. [Obtained from Massachusetts Department of Natural Resources, Division of Marine Fisheries, Boston, Massachusetts.]

Gooch, J. L., and T. J. M. Schopf, 1969. Genetic studies on marine species of the Phylum Ectoprocta. *Biol. Bull.*, **137**: 400–401.

Gooch, J. L., and T. J. M. Schopf, 1970. Population genetics of marine species of the Phylum Ectoprocta. *Biol. Bull.*, **138**: 138–156.

Hastings, A. B., 1968. Some type and other specimens of species involved in the problem of *Stylopoma* Levinsen (Polyzoa). *Bull. Brit. Mus. (Natur. Hist.) Zool.*, **16**: 355–364.

Johnson, C. W., 1934. List of marine mollusca of the Atlantic Coast from Labrador to Texas *Proc. Boston Soc. Natur. Hist.*, **40**: 1–204.

Koehn, R. K., 1969. Esterase heterogeneity: dynamics of a polymorphism. *Science*, **163**: 943–944.

Koehn, R. K., 1970. Functional and evolutionary dynamics of polymorphic esterases in Catostomid fishes. *Trans. Amer. Fish. Soc.*, **99**: 219–228.

Manwell, C., and C. M. A. Baker, 1970. *Molecular Biology and the Origin of Species.* University of Washington Press, Seattle, 394 pp.

Milkman, R., 1967. Genetic and developmental studies on *Botryllus schlosseri. Biol. Bull.*, **132**: 229–243.

Milkman, R., and L. Beaty, 1970. Large-scale electrophoretic studies of allelic variation in *Mytilus edulis. Biol. Bull.*, **139**: 430.

Powell, N. A., 1970. *Schizoporella unicornis*—An alien bryozoan introduced into the Strait of Georgia. *J. Fish. Res. Board Canada*, **27**: 1847–1853.

Sabaddin, A., 1959. Analisi genetica del policromatismo di *Botryllus schlosseri* (Pallas) Savigny (Ascidiacea). *Boll. Zool.*, **26**: 221–243.

Schopf, T. J. M., and J. L. Gooch, 1970. A natural experiment in the population genetics of a marine ectoproct suggesting changes in gene frequencies with changes in temperature. *Biol. Bull.*, 139: 435.

Schopf, T. J. M., and J. L. Gooch, 1971. Gene frequencies in a marine ectoproct: a cline in natural populations related to sea temperature. *Evolution*, **25**: 286–289.

Selander, R. K., S. Y. Yang, R. C. Lewontin and W. E. Johnson, 1970. Genetic variation in the Horseshoe Crab (*Limulus polyphemus*), a phylogenetic "relic." *Evolution*, **24**: 402–419.

West, D. A., 1964. Polymorphism in the isopod *Sphaeroma rugicauda. Evolution*, **18**: 671–684.